7 TOXICOKINETICS OF NICOTINE-DERIVED NITROSAMINES IN THE RAT

B.L. Pool-Zobel, U. Liegibel, P. Schmezer, R.G. Klein
Institute for Toxicology and Chemotherapy, German Cancer Research Center,
Im Neuenheimer Feld 280, 6900
Heidelberg, F.R.G.

The two tobacco specific nitrosamines NNK (4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone) and NNN (nitrosonornicotine) are important toxic ingredients of tobacco and tobacco smoke. They are formed from nicotine during curing and fermentation of tobacco leaves by nitrosation. Hereby, the nitrate concentration plays a predominant role for their formation (Fischer et al., Carcinogenesis 10, 1989). Relatively high concentrations have been found in tobacco (up to 8850 p.p.b. NNN and 1400 ppb NNK) and in tobacco smoke (up to 625 ng/cig NNN and up to 432 ng/cig NNK) by Fischer et al. (Carcinogenesis 10, 1989a; 1989b). Furthermore NNN and NNK are potent carcinogens which may induce tumors locally or systemically. Accordingly, NNN has been found to induce tumors of the nasal cavity and oesophagus of rats after s.c. and oral application, whereas nasal cavity, lung, liver and pancreas tumors were induced by NNK (rev. Hecht & Hoffman, Cancer Surveys 8, 1989). Therefore these nitrosamines could well be responsible also for the human malignant tumors which occur locally in respiratory and upper digestive tract, or systemically in bladder, renal pelvis and pancreas (IARC Monograph, Vol 38) following uptake of tobacco and tobacco smoke related products.

It was the aim of this study to elucidate in which manner the active metabolites of NNN and NNK contribute to the systemic carcinogenic properties of tobacco related products. The experimental approach was to assess toxic and genotoxic effects by N-nitrosodimethylamine (NDMA), NNK and NNN in different organs of the rat. For this, induced DNA single strand breaks (SSB) were first determined in hepatocytes *in vitro* to comparatively establish the genotoxic potential of the compounds. Furthermore SSB were assessed in isolated cells of different organs following single *p.o.* application and 1 h exposure to the test chemicals. Oral application was chosen as the first exposure route, since it is relevant for the chewing and snuff dipping use of tobacco. So far, with the alkaline elution technique of Kohn et al. (Biochemistry 15, 1976) it has been found that all three compounds are genotoxic *in vitro* (NDMA = NNK> NNN) within the concentration range of 6.25 - 25 umoles/ml/10^6 hepatocytes.

Ex vivo, NDMA is a potent inducer of DNA SSB in liver (>0.1 mg/kg), lung, kidney and lymphocytes (>2 mg/kg). No genotoxicity was observed in testes or thymus. NNK induces SSB at 35 and 50 mg/kg in the liver and lower doses are being assessed. 25-100 mg /kg NNN was not genotoxic in the liver. This dose range is only 2.5-10% of its reported LD_{50}, but is equitoxic to the very genotoxic dose range of 1-4 mg/kg NDMA. We are presently evaluating NNK and NNN in additional organs (e.g. oesophagus) to identify susceptible target tissues. Since this cell yield is too low to perform the alkaline elution, genotoxic effects are detected by other techniques. Accordingly, first results with a single cell analysis of DNA damage by *in situ* nick translation (Anai et al., Cancer Letters 40, 1988) will be presented. This type of approach in toxicokinetics may aid not only in elucidating the role of individual compounds of the complex carcinogenic mixtures tobacco and tobacco smoke, but may also serve as a useful modell system for studying specific mechanisms.